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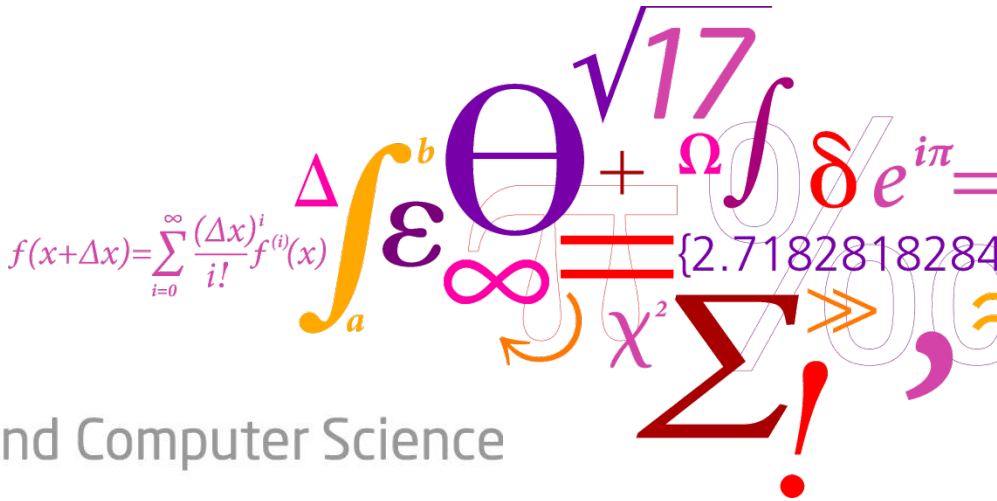

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Infectious risk factors for individual postweaning multisystemic wasting syndrome (PMWS) development in pigs from affected farms in Spain and Denmark

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Entered project group after all experiments had been performed

Postweaning Multisystemic Wasting Syndrome (PMWS)

- Multifactorial syndrome for pigs.
- Not a ‘disease’, but an immune system breakdown.
- Clinical signs:
  - Weight loss;
  - Enlarged lymph nodes;
  - Respiratory distress;
  - Some times diarrhea and jaundice;
  - Death/’wasting’.
- VERY costly (Armstrong and Bishop 2004); fattening pigs that do not put on weight or die are of course problematic.
- Cause: Unknown. Associated with Porcine Cirvovirus type 2 (PCV2), but the exact association is not clear.

Cause of PMWS and this study

- In general, Unknown (at least for what regards PMWS).
- Associated with Porcine Cirvovirus type 2 (PCV2), but very difficult to reproduce in controlled studies with PCV2 infections alone.

Cause of PMWS and this study

- In general, Unknown (at least for what regards PMWS).
- Associated with Porcine Cirvovirus type 2 (PCV2), but very difficult to reproduce in controlled studies with PCV2 infections alone.
- Meta analysis (Thomás 2008) suggests that PMWS may be reproduced through infection with PCV2 and co-infections with other pathogens.
- Thus, in the study that this analysis is based on, we looked at measures for infections with PCV2 and the following pathogens:
  - Porcine parvovirus;
  - Svine influenza virus, strains H1N1 or H3N2;
  - *Lawsonia intracellularis*;
  - Porcine Reproductive and Respiratory Syndrome virus, European and American variant;
  - Aujesky's disease virus;
  - Mycoplasma hyopneumonia;
  - Salmonella Spp.

Purpose of Study Analysis

- To uncover the role of specific pathogens in the development of PMWS

Working Hypotheses:

1. The development of antibodies towards pathogens through seroconversion after infection **increases** the risk of developing PMWS.

2. Immunity inherited from the mother animal has a **reducing** effect on the risk of developing PMWS.

PMWS Diagnosis

- Presence of compatible clinical signs
- Moderate to severe lymphocyte depletion
- Granulomatous inflammation in lymphoid tissues
- Detection of moderate to high amount of PCV2 within these lesions

(Segalés et al., 2005; Sorden, 2000).

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(Segalés et al., 2005; Sorden, 2000).

Not possible to diagnose without an autopsy.

Data material

- Antibody measurements were taken at pre-specified time points;
- Animals were selected in Denmark and Spain after clinical signs (cases) and youthanized;
- Age-matched controls were selected (fewer) and youthanized;

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- Some of the ‘cases’ turned out not to be PMWS diagnosed…

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- Age-matched controls were selected (fewer) and youthanized;
- However, the ‘cases’ were not diagnosed at selection, as this requires an autopsy.
- Some of the ‘cases’ turned out not to be PMWS diagnosed…
- And some of the controls could turn out to be cases, had they been allowed to live on…

Solution: Survival Analysis Framework

- PMWS status at autopsy; death/failure is PMWS development, if not observations are censored at autopsy. Wasting non-PMWS animals excluded but used for control.

- Covariates: Herd ID, and:

- Longitudinal measurements of antibody titres / OD% for the following pathogens on 135 pigs (DK), 120 pigs (E) :

- Porcine parvovirus (**PPV**);
- Porcine cirvovirus type 2 **(PCV2**);
- Svine flu H1N1 or H3N2 (**SIV**);
- Lawsonia intracellularis (**LAW**);
- European Porcine Reproductive and Respiratory Syndrome virus (**PRRSV.E**);
- American Porcine Reproductive and Respiratory Syndrome virus (**PRRSV.U**).

Relations to Working Hypotheses

- No direct measure of time for seroconversion;
- No direct measure of maternal immunity (mother animals cannot be used due to cross-fostering).

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Construction of such measures necessary

Maternal immunity

- Not possible to use values for mother animals due to *cross-fostering*: Piglets are taken from one mother animal and laid at another, to maximize piglet survival.

- Maternal immunity estimated as the *maximum registrated antibody measurement in the first three weeks of life*.

Seroconversion Times

- Pathogen antibody measurements declines with time, until an infection makes it rise again.

- The time point for seroconversion is the point in time where antibody concentration increases after the initial decline, without delay.

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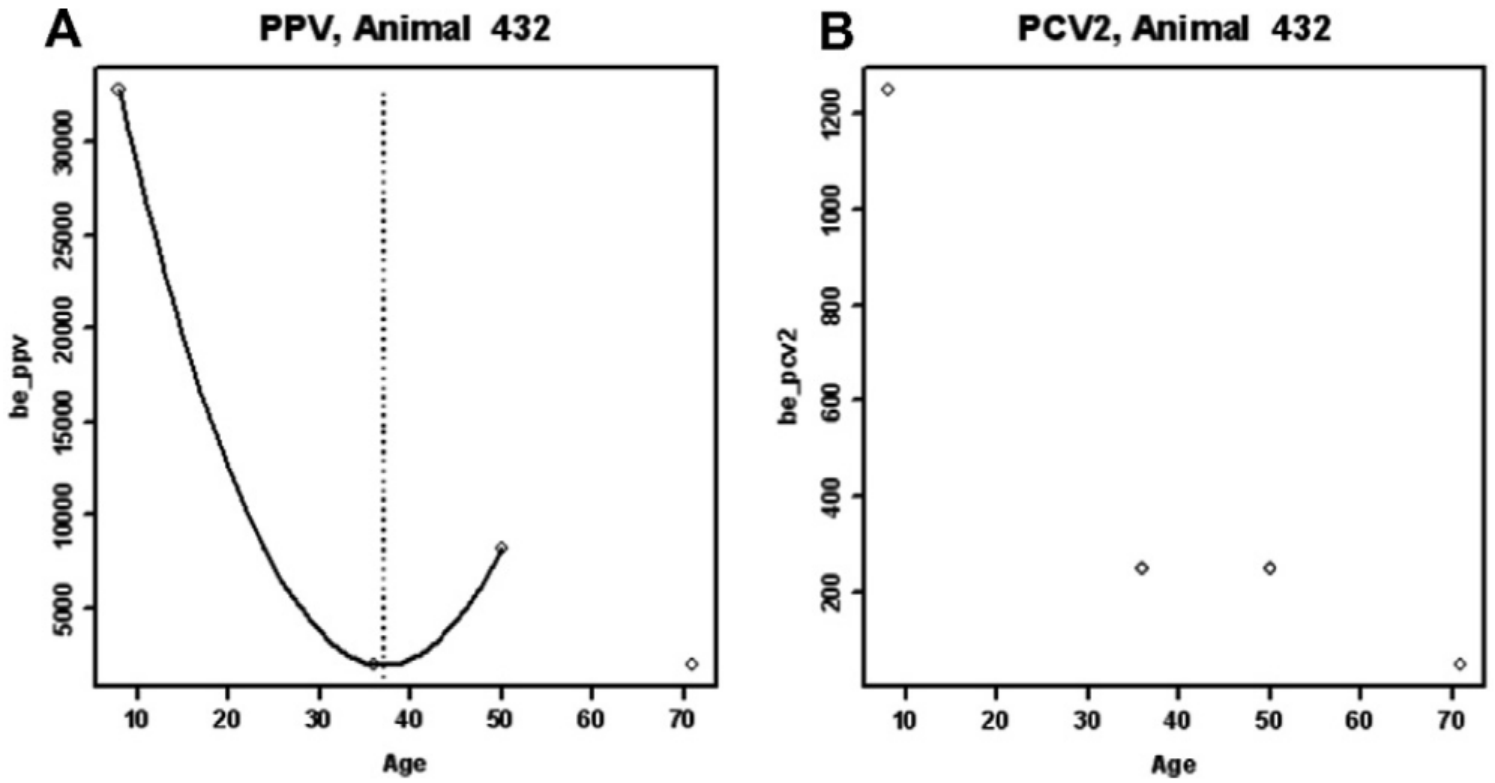
- Pathogen antibody measurements declines with time, until an infection makes it rise again.
- The time point for seroconversion is the point in time where antibody concentration increases after the initial decline, without delay.
- To estimate this estimate from only a few observations, the antibody concentration progress is estimated through regression of 2^{nd} order polynomials on the longitudinal data.
- The Seroconversion Time is estimated as the time point corresponding to the vertex of the parabola.

Seroconversion and No Seroconversion



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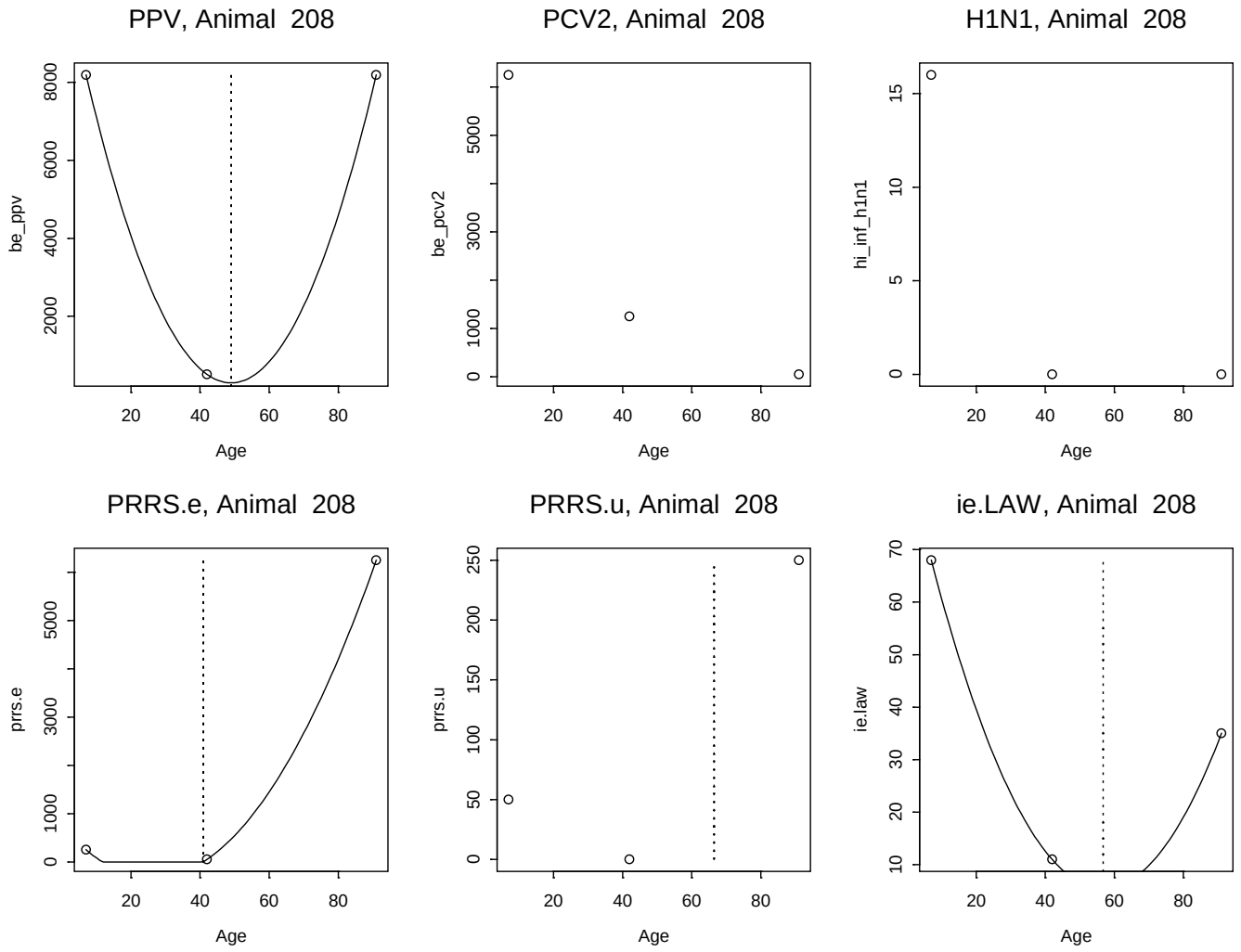


Survival Analysis

- P(PMWS case in [t:t+Δt) | no case at t) ≈ λ(t) Δt
- Cox' Proportional Hazards model for animal *i*:

$$\lambda_i(t) = \lambda_0(t)\exp(\beta_1 X_{i1}(t) + \beta_2 X_{i2}(t) + \ldots + \beta_k X_{ik}(t))$$

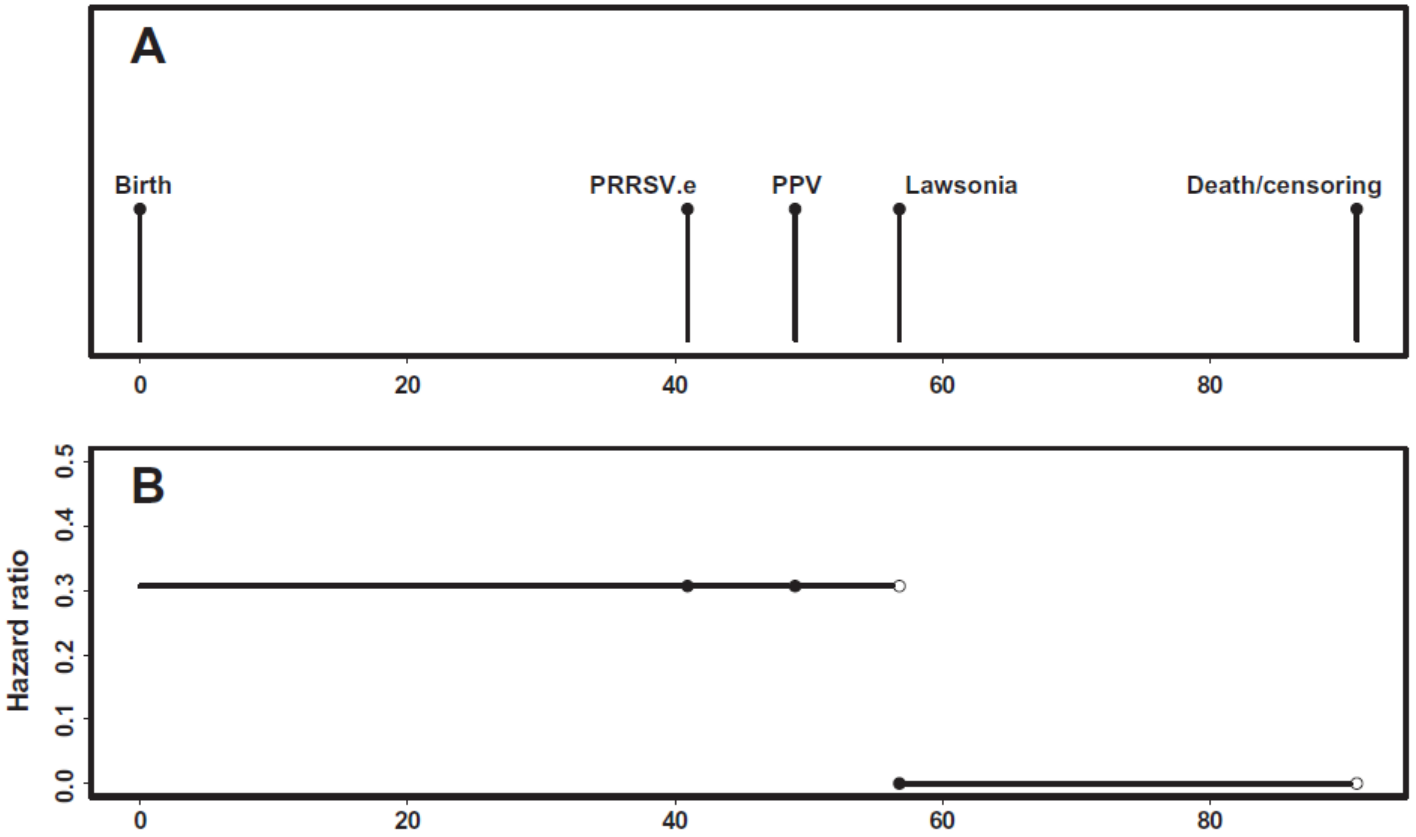
- Covariates are maternal immunity (not time dependent), seroconvertion times. and interactions within and between these (time dependent).
- λ_0 is non-parametric and not modelled.

P(PPV seroconverted animal case in [t:t+Δt) |not case at t)/
P(non-seroconverted animal case i [t:t+Δt) | not case at t)
$= \exp(\beta_{ppv})$

if all other characteristics match;

- Relative risks only because λ_0 is not modeled.

Exemplified Hazard Ratio Development



Sensitivity Analysis

- In order to contemplate the impact of the built-in impreciseness of the estimates of seroconversions, a sensitivity analysis was carried out after model reduction. Gaussian noise was added to the seroconversion times considering that 95% of the new seroconversion times should be within one week of the original estimates.

- Noise addition and model reduction was performed 20 times;

- In order to be rendered truly significant a significant factor must appear in at least half of the analyses' final models.

Estimation Method – Few Data, Many Covariates

1. Include all seroconversions and interactions. Reduce.
2. Include each maternal immunity in a pre-specified sequence, and all interactions with factors in current model. Reduce.
3. Include all apparently non-significant factors again through forward selection, one at a time. Reduce.
4. Repeat steps 1-3 until no changes results; ie no increase in Cox' partial likelihood.

Results

- DK:
- Seroconversion against **LAW**;
- Seroconversion against **PRRSVe**;
- maternal immunity against **PCV2;**
- maternal immunity against **LAW**.

- Spain:

- Maternal immunity against **LAW**, **PCV2**, **PPV**, **PRRSV** and **SIV**.

Results

- DK:
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Results

Covariate	Estimated $\beta \pm 1.96$SE	p^*
(A)		
Law	10.322 ± 7.10	0.002
log(mat.pcv2)	−0.561 ± 0.26	<0.0001
log(mat.law)	−4.02 ± 2.73	0.0005**
Covariate	Estimated $\beta \pm 1.96$SE	p^*
(B)		
log(mat.law)	−11.46 ± 6.49	0.002
log(mat.pcv2)	7.26 ± 7.22	0.007
log(mat.pcv2)^2	−0.72 ± 0.60	0.008
log(mat.ppv)	11.29 ± 6.49	<0.0001
mat.prrsv	11.08 ± 5.97	0.0001
mat.siv	64.87 ± 47.39	<0.0001
log(mat.law): log(mat.pcv2)	0.64 ± 0.60	0.03
log(mat.law): mat.prrsv	−2.64 ± 1.46	0.0003
log(mat.law): mat.siv	7.94 ± 4.58	0.0008
log(mat.ppv): mat.siv	−13.46 ± 6.87	<0.0001

* Tests of main effects includes removal of interaction terms.

** The effect of mat.law extends to lawsonia sero-converted animals only.

Significant Impact?

- Create an index *I* for animals with maternal immunity around average, by differentiating the log of the Cox PH after the covariates (in distributional sense for seroconversions) for factors interacting, and take means of these. For factors not interacting, the index *I* is the parameter estimate.

$$\text{(a) } I(\text{law}|\text{Danish}) = \beta_{\text{law}} + \beta_{\text{law:mat.law}} \cdot \text{mean}(\log(\text{mat.law}))$$

$$\begin{aligned}\text{(b) } I(\text{mat.law}|\text{Spanish}) = \beta_{\text{mat.law}} &+ \beta_{\text{mat.law:mat.pcv2}} \cdot \text{mean}(\log(\text{mat.pcv2})) \\ &+ \beta_{\text{mat.law:mat.prrsv}} \cdot \text{mean}(\text{mat.prrsv}) \\ &+ \beta_{\text{mat.law:mat.siv}} \cdot \text{mean}(\text{mat.siv})\end{aligned}$$

$$\begin{aligned}\text{(c) } I(\text{mat.pcv2}|\text{Spanish}) = \beta_{\text{mat.pcv2}} &+ 2\beta_{\text{mat.pcv2.2}} \cdot \text{mean}(\log(\text{mat.pcv2})) \\ &+ \beta_{\text{mat.pcv2:mat.siv}} \cdot \text{mean}(\text{mat.siv})\end{aligned}$$

$$\text{(d) } I(\text{mat.ppv}|\text{Spanish}) = \beta_{\text{mat.ppv}} + \beta_{\text{mat.ppv:mat.siv}} \cdot \text{mean}(\text{mat.siv})$$

$$\text{(e) } I(\text{mat.prrsv}|\text{Spanish}) = \beta_{\text{mat.prrsv}} + \beta_{\text{mat.prrsv:mat.siv}} \cdot \text{mean}(\text{mat.siv})$$

$$\begin{aligned}\text{(f) } I(\text{mat.siv}|\text{Spanish}) = \beta_{\text{mat.siv}} &+ \beta_{\text{mat.pcv2:mat.siv}} \cdot \text{mean}(\log(\text{mat.pcv2})\end{aligned}$$

Indexes; Values and Significances

Pathogen type	Covariate type	Calculated index ± 1.96SE	Hazard ratio* (CI)	*p* (Chisq)
I (law|Danish)	Seroconversion	−1.45 ± 1.44	0.23 (0.06;0.99)	0.05
I (mat.law|Spanish)	Maternal immunity	−0.29 ± 0.64	0.75 (0.39;1.42)	0.63
I (mat.PCV2|Spanish)	Maternal immunity	−2.75 ± 1.05	0.06 (0.02;0.18)	<0.0001
I (mat.PPV|Spanish)	Maternal immunity	−3.35 ± 1.80	0.04 (0.01;0.21)	<0.0001
I (mat.PRRSV|Spanish)	Maternal immunity	2.32 ± 1.23	10.18 (2.97;34.81)	0.0002
I (mat.SIV|Spanish)	Maternal immunity	−4.15 ± 4.14	0.02 (0.00;0.99)	0.05

* For continuous covariates, the hazard ratio is per increase of 1.

Indexes; Impact

Covariate	Survival analysis	Single-term analysis
(A)		
Seroconversion law	Protecting	Not significant
Seroconversion PCV2	Not significant	Not significant
Seroconversion PPV	Not significant	Not significant
Seroconversion SIV	Not significant	Not significant
Seroconversion PRRSVe	Not significant	Not significant
Seroconversion PRRSVu	Not significant	Not significant
Maternal law	Protecting*	Not significant
Maternal PCV2	Protecting	Protecting
Maternal PPV	Not significant	Not significant
Maternal SIV	Not significant	Aggravating
Maternal PRRSVe	Not significant	Not significant
Maternal PRRSVu	Not significant	Not significant

Covariate	Survival analysis	Marginal model
(B)		
Seroconversion PCV2	Not significant	Not significant
Seroconversion PPV	Not significant	Not significant
Seroconversion SIV	Not significant	Not significant
Seroconversion PRRSV	Not significant	Not significant
Seroconversion Salmonella	Not significant	Not significant
Maternal law	Not significant	Not significant
Maternal PCV2	Protecting	Not significant
Maternal PPV	Protecting	Not significant
Maternal PRRSV	Aggravating	Aggravating
Maternal SIV	Protecting	Not significant

* Applied to *Lawsonia intracellularis* seroconverted animals only.

Working Hypotheses

1. The development of antibodies towards pathogens through seroconversion after infection **increases** the risk of developing PMWS.

In CONTRAST to results for LAW

2. Immunity inherited from the mother animal has a **reducing** effect on the risk of developing PMWS.

CONFIRMED for

» PCV2, LAW in Denmark,

» PCV2, PPV and SIV in Spain.

CONTRASTED for

» PRRSV in Spain.

Possible Explanations

- Seroconversion towards LAW:
  - We DON'T observe infections but merely seroconversions;
  - Animals may be infected but unable to seroconvert due to progressing immune defiency;
  - That animals seroconvert may indicate a functioning immune system, which overshadows the weakening effect of infection with *Lawsonia intracellularis*.
- Lack of seroconversion effects for Spanish data:
  - Strongly heterogeneous population.
- Maternal immunity in Spanish data:
  - Consistent with litterature for PCV2, PPV, SIV (see refs in paper).
  - PRRSV results may be explained by the heterogeneous population; thus maternal immunity may indicate high presence of PRRSV which is known from the litterature as a PMWS trigger.

Spanish Data and Seroconversions

PPV	sero-converted	not sero-converted	sum
case	2	45	47
not case	6	66	72
sum	8	111	119

PCV2	sero-converted	not sero-converted	sum
case	40	7	47
not case	68	4	72
sum	108	11	119

PRRSV	sero-converted	not sero-converted	sum
case	2	45	47
not case	12	60	72
sum	14	105	119

SIV	sero-converted	not sero-converted	sum
case	28	19	47
not case	59	13	72
sum	87	32	119

- Difficult to identify effects from such distributions of seroconversions.
- But the lack of time-dependent covariates means that it is sensible to compare risks and frequencies through grouping. They agree...

Conclusion

- Protective effect of seroconversion against law (DK).
- Protective effects of maternal immunity against PCV2 (DK,E), PPV (E) and SIV (E).
- Aggravating effect of maternal immunity against PRRSV.

- All effects may be compatible with present knowledge except law, where seroconversion increases the risk when maternal immunity is low; ie the disease triggers PMWS unless maternal immunity is high. First report on this.
- The level of detail in the analysis is new compared to existing knowledge.

- Care should be taken when generalizing spanish results due to population heterogeneity.

- Further work should include PCR data to counter indirect detection of infections.